OBSERVATIONS ON THE LIFE CYCLE OF *STICTODORA LARI* (TREMATODA: HETEROPHYIDAE).

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(Fifteen Text-figures.)

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Synopsis.

1. The life cycle of *Stictodora lari* includes passage through an operculate gastropod, *Velacumantus australis,* as first intermediate host and through several species of fish as second intermediate hosts. The latter include mullet, gobies and other small fish living in weeds growing in shallow brackish water, the habitat preferred by *V. australis.*

2. Mature parasites occur naturally in *Larus novae-hollandiae* and other fish-eating birds. They can also be reared in cats.

The present paper is one of several dealing with cercariae of estuarine molluscs in Sydney District; titles of these papers are given in the bibliography (Bearup, 1956, 1958, 1960).

The main collecting sites for vectors have been shallow sandflats in Narrabeen Lagoon, a few miles north of Sydney. This brackish lagoon receives several small freshwater streams and empties into the sea through a narrow channel often blocked by sand during rough weather. Estimations of the percentage of chloride in the water over the chief collecting area varied between 1·84 in dry weather and 0·89 after heavy rain. Some sandflats have heavy growths of *Zostera* weed which is the favoured habitat of *Velacumantus australis* (= *Pyrazus australis*), the main vector of *Stictodora* cercariae.

Materials and Methods.

Collections of *V. australis* were made at about monthly intervals and the incidence was calculated by crushing 100–200 of them. Living cercariae were collected from single snails isolated in brackish water in small glass tubes. The cercariae are easily distinguished from other cercariae in *V. australis* by their rapid swimming movements and by the fin-fold on the tail.

Fish were infected by exposing them to naturally emerged cercariae in Petri dishes. The guppies, *Lebistes ? reticulatus,* were aquarium bred, the *Gambusia* were taken from freshwater ponds but showed no natural infections with trematodes. Both types were conditioned to brackish water by several additions of sea-water, over several hours. Infections were only successful in brackish water; in fresh water the cercariae lost motility and were soon dead.

Adults were recovered from young seagulls (*Larus novae-hollandiae*) which had been fed on beef heart since they were fledgelings. Kittens, also, were easily infected.

Observations.

Details of the life cycle are incomplete because free miracidia have not been obtained, nor has an experimental infection of the snail been studied. The youngest larvae found, in natural infections, were small colourless rediae in squash preparations of the mantle and digestive gland and no clear evidence of two generations of rediae was found.

Ova (Fig. 1).

Mature ova were thick-shelled, brown, elliptical to oval, with the operculum poorly defined. Ova in the lower uterus or in faeces measured 0·033–0·035 by 0·014–0·020 mm. (average 0·034 × 0·019 mm.) and contained a miracidium, probably mature, although no ciliary movement was seen. Details of the larva were obscured by the thick brown shell.

Rediae (Figs 2, 3).

The youngest larvae found were colourless rediae in the mantle and digestive gland (0·148 by 0·022 mm.) with a gut extending nearly the full length, a pharynx as wide as the body, and germinal tissue filling the posterior end (Fig. 2). The pharynx was mobile and when retracted the anterior body wall formed an outwardly-directed lip. Infections which had mature cercariae also had all stages of developing rediae, the latter varying in size up to 1·1 mm. The large ones contained many germ balls, developing cercariae and usually several advanced cercariae with eyespots but of smaller size than mature ones, and lacking fin-folds on the tail (Fig. 3). No collar or posterior projections were found in young or old rediae. Older rediae were lightly coloured with patches of yellowish-brown pigment in the body wall and in the gut. The gut becomes relatively smaller in large rediae and is eventually confined to a small space behind the pharynx. No birth pore was found.

Neither rediae nor the oldest cercariae within them show more than occasional sluggish movements. The cercariae apparently mature in the digestive gland and, when the surface of this organ is broken, very active cercariae escape in large numbers.

Cercariae (Figs 4, 9).

Small, monostome cercariae of the pleurolophocerca type. Average dimensions (in millimetres) of 10 cercariae killed in hot water: body, 0·219 long by 0·087 wide; tail, 0·429 long by 0·031 wide at base; eyespots 0·061 from anterior end; oral sucker, 0·034 long by 0·031 wide. Cercariae recently killed in hot formalin are smaller; body 0·202 by 0·071; tail, 0·415 by 0·027. Body shape roughly pyriform when at rest. Cuticle thin, covered with rows of extremely fine spines. No sensory bristles found. Oral sucker modified to a rounded protrusible penetrating organ ("anterior organ") with a subterminal mouth on it.

Anterior to the mouth opening is a small pit containing a group of spines with fine lanceolate tips. They are hard to find and in the cercaria are usually seen as a double row of 7 or 8 tips supported by parallel rods. A better view of them in a young metacercaria (2 hours after exposure) showed 16 spines in a circle projecting from the pit, like stamens from a flower (Fig. 9).

Paired eyespots large, at about one-fourth of body length from anterior end; scattered pigment granules around the eyespots and along the lateral borders of the cercaria.

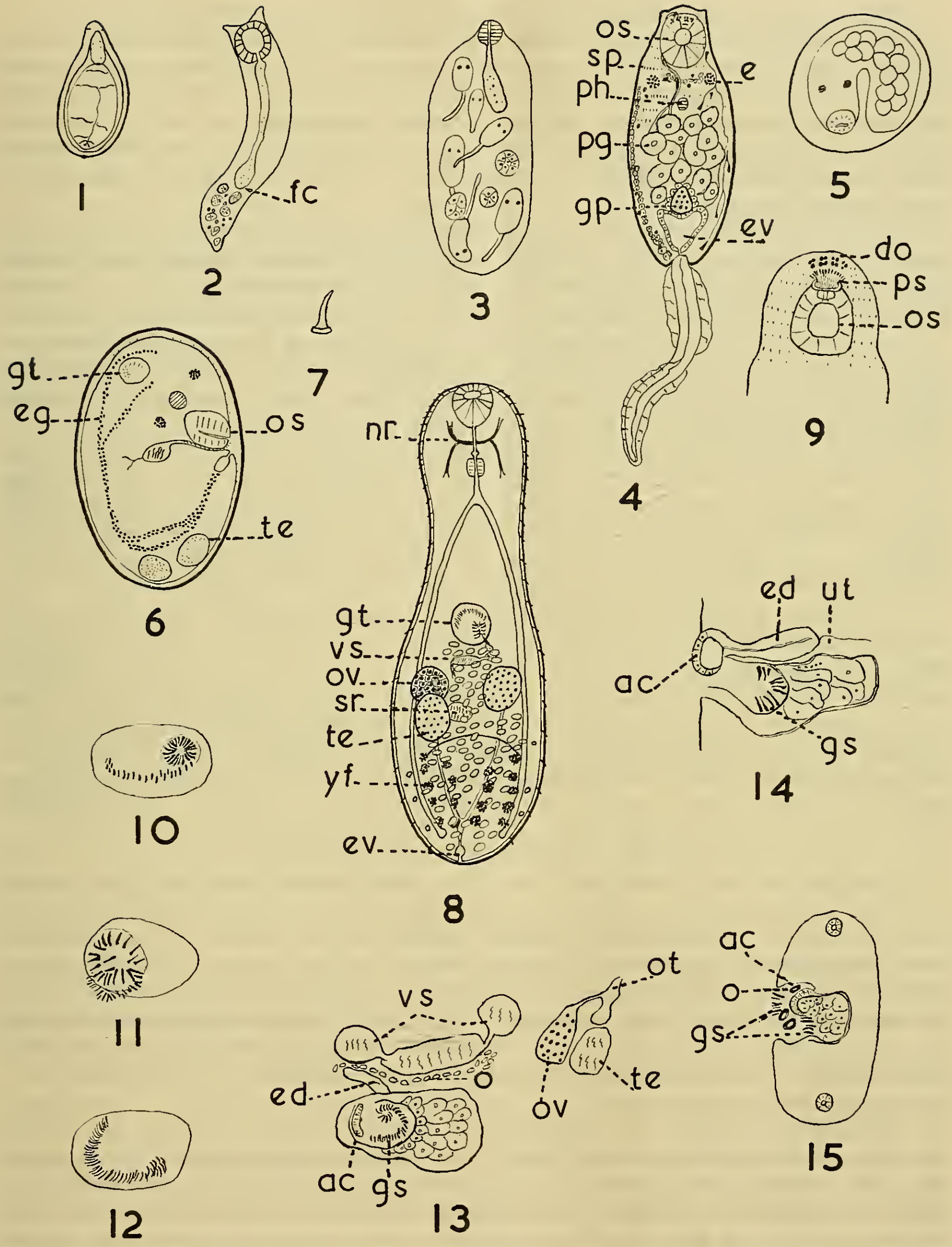
Tail is a powerful swimming organ with two sets of delicate, cuticular fins. Lateral fins extend about 40% of tail length from body and the dorso-ventral fin passes around the tip and extends to about 60% of the tail length. The fins are thrown into small convolutions by the contraction of the tail. Tip of tail bent laterally.

Centre of body occupied by 14 large penetration gland cells with smooth or slightly wavy outlines, large conspicuous nuclei, and finely granular protoplasm staining red with neutral red.

From these cells, lateral ducts go forward in two groups between the eyespots and then diverge; they pass around the dorsal side of anterior organ to open at its anterior border. The inner group consists of four and the outer group of three ducts on each side. Small cells, probably cystogenous cells, line the body just below the cuticle. Pharynx poorly defined; oesophagus and gut not found.

Genital primordium a compact triangular cell mass in front of the bladder.

Excretory system of mesostomate type; consists of large thick-walled vesicle of variable shape but Y-shaped when cercaria at rest; walls of cuboid epithelium. Posterior canal runs into tail; in immature cercariae it divides near the base into two vessels which open laterally. In the body the main canals leave the vesicle antero-laterally and branch into two secondary ones each draining three flame cells. Total number of flame cells in young cercariae is 12 with a formula of 2(3 + 3). The excretory system of mature cercariae is obscured by granules of the penetration gland cells and methods which are often useful (coverslip pressure, examination in neutral red, serum or urine or in young metacercariae) do not make it visible.



Figures 1-15.—Stages in life cycle of *Stictodora lari*. 1, ovum; 2, young redia, from mantle of *V. australis*; 3, older redia, from digestive gland, *V. australis*; 4, cercaria, naturally emerged from *V. australis* (excretory system, drawn from immature cercaria, is superimposed); 5, young metacercaria, 18 hours after penetration, *Gambusia*; 6, metacercaria, 26 days after penetration, *Gambusia*; 7, gonotyl spine; 8, adult, from experimental feeding, *Larus*; 9, cercaria, anterior end, showing penetration spines; 10, 11, 12, gonotyl spines of 3 adults from one experimental infection of *Larus*; 13, adult, genital sac and other genitalia, flattened under coverslip; 14, 15, T/S through genital sac. AC, acetabulam; DO, openings of ducts—penetration glands; E, eyespots; ED, ejaculatory duct; EG, excretory granules; EV, excretory vesicle; FC, flame cells; GP, genital primordium; GT, gonotyl; GS, gonotyl spines; NR, nerve ring; O, ova; OT, ootype; OS, oral sucker; OV, ovary; PG, penetration gland cells; PH, pharynx, PS, penetration spines; SR, receptaculam seminis; TE, testis; UT, uterus; VS, vesicula seminis; YF, yolk follicles.

Nerve ring indistinct, about half-way between pharynx and anterior organ.

The cercariae are active swimmers; two or three seconds of vigorous swimming alternate with longer periods of rest, which vary up to about 10 seconds. While swimming they rise slightly in the water but fall slowly to the bottom during rest. The cercariae are not adapted to creeping and they have difficulty in moving on a glass surface because the "anterior organ" does not seem to act as a sucker and there are no other organs of adhesion. They were attracted to the lighted side of the vessel but not to the surface of the water.

Cercariae lived for 18–24 hours in brackish waters of salinities varying from 3% down to 0·5% sodium chloride. In pond water (0·01% sodium chloride) they stopped swimming immediately and died within an hour. Low temperatures (5° C.) prolong their life and infected snails can be stored for a month in the refrigerator.

Cercariae of this type are common throughout the year in *V. australis* in Narrabeen Lagoon, where the incidence sometimes rose above 30% and would average about 18% (645 in 3,565 *V. a.*), but with no clear seasonal variation. The percentages of infections at intervals over three years are given in brackets: August 1956 (12); x.1956 (15); xii.1956 (7); i.1957 (16); v.1957 (17); ix.1957 (33); xi.1957 (34); i.1958 (43); v.1958 (8); ix.1958 (12); xi.1958 (15); iii.1959 (38). Infection rates were low in young *V. australis* under 25 mm. long and were highest in the 30–34 mm. group; they were also higher in weedbeds not swept by the tide than in those bordering the channels. Double infections were common with the forked-tail cercariae of *Austrobilharzia terrigalensis*, occasionally with *Echinostoma* cercariae, and once a triple infection was found.

V. australis is common on *Zostera* flats in estuaries from about 500 miles south to 500 miles north of Sydney and *Stictodora* cercariae are present throughout the range. Other snails sharing the habitat were usually free of these cercariae, but one infection was found in each of two species, *Pyrazus ebeninus* and *Parcanassa ellani.* The names and numbers of molluscs examined included *Pyrazus ebeninus* Bruguière (73); *Parcanassa ellana* Iredale (326); *Salinator fragilis* Lamarck (339); *Austropyrgus ruppiae* Hedley (1,023); *Austrocochlea obtusa* Dillwyn (143); *Bembicium auratum* (Quoy & Gaimard) (84); *Thalotia comtessei* Iredale (35); *Eumarcia ? fumigata* (64).

Cysts and Metacercariae (Figs 5, 6).

Metacercarial cysts containing *Stictodora* metacercariae were found as natural infections in several kinds of small fish that frequent the weedbeds, including species of mullet, gobies, leather-jackets, pipefish, toadfish and hardyheads. Those identified were: *Favingobius lateralis obliquus* McCulloch and Ogilby; *Atherinosoma microstoma* (Gunther); *Urocampus carinorostris* (Castelnau); *Waiteopsis paludis* Whitley; *Mugil* sp. and *Gambusia affinis* Baird and Girard. *Gambusia* sometimes migrates into brackish water and shelters in the weedbeds; of 24 collected from this habitat, 8 had *Stictodora* metacercariae. The same species from a suburban freshwater pool was found to be free of trematode metacercariae.

Freshwater *Gambusia* and *Lebistes* were infected in the laboratory by exposing them in brackish water in small glass Petri dishes to large numbers of naturally emerged cercariae. The penetration of cercariae into the fish could then be watched under a dissecting microscope. The cercariae do not seem to be attracted by the proximity of the fish, but once they make contact the anterior organ is directed towards the skin and is kept closely applied by vigorous lashing of the tail. The cercarial body then bends through 90° to become parallel to the side of the fish and moves to and fro through a quarter-circle, apparently using the spines of the anterior end to bore a hole through the skin. Once penetration is effected the tail falls off.

In ten minutes penetration of the body of the fish was complete. Those cercariae which entered the fins did not remain there but moved towards the body and came to rest in the nearby muscles. Two hours after exposure many cercariae were enclosed in a thin transparent cyst wall and were bent in a semicircle within it; the cyst at this stage measured 0·18 mm. across. The most prominent feature of the young larva was the dark mass of granules in the excretory bladder.

The eyespots and gland cells of the cercaria gradually disappeared and were replaced by adult characters. At 26 days the rounded cysts had diameters up to 0·4 mm. and enclosed metacercariae 0·9–1·0 mm. long by 0·18–0·20 mm. wide with an ovary, testes, gonotyl with rudimentary spines and a complete digestive system with caeca reaching almost to the posterior end. Rows of fine backwardly-directed spines surrounded the body as far back as the gonotyl; posteriorly they got fewer and finally disappeared. At this stage, perhaps earlier, they were infective for seagulls and kittens.

Stictodora cysts in naturally acquired infections were scattered through the body muscles with an odd one in the abdominal viscera, especially in the liver. Cysts were also found free in the body cavity of pipefish, but they were always small and immature. Heavily infected pipefish with dark skin (*Urocampus*) show many small, round, depigmented patches, probably each representing the point of entry of a cercaria.

Feeding experiments to experimental hosts and the results at postmortem follow. Naturally infected fish were all netted from Narrabeen Lagoon, from weedbeds sheltering infected *Velacumantus*. The dates of multiple feedings and of postmortem are given.

A.—Young gull, fed cysts from experimentally infected *Gambusia* after 26 days' development in the fish. Seventeen fish had 22 cysts, the largest measuring 0·45 × 0·49 mm. Thirteen days later, four *Stictodora* recovered.

B.—Young gull, fed cysts from naturally infected *Gambusia*; twenty-three days later, 46 *Stictodora* recovered.

C.—Kitten, fed *Favingobius*, natural infections, 25.xi and 26.xi; forty *Stictodora*, 8.xii.

D.—Kitten, fed small mullet and *Atherinosoma*, natural infections, 7.x, 10.x; seven *Stictodora*, 28.x.

E.—Kitten, fed cysts dissected from toadfish and *Atherinosoma*, natural infections, 11.v, 23.v; five *Stictodora*, 2.vi.

F.—Kitten, fed *Atherinosoma*, natural infection, 4.xi, 12.xi; two *Stictodora*, 14.xi.

Young (2 days) chickens and pigeons were also fed with cysts, but no adults were recovered.

No trematode other than *Stictodora* was present in any of the experimental hosts and a careful examination of the specimens showed no specific differences. The name of the species is discussed later.

Adult (Fig. 8).

The description which follows is based on the examination of many living worms as well as fixed preparations stained with carmine and mounted in balsam. Measurements of anatomical structures in the fixed specimens were about 10% lower than in living worms under slight coverslip pressure.

Body flattened dorso-ventrally, constricted about the middle, 1·15–1·33 × 0·26–0·44 mm. at the level of the testes (average of 10 living worms, 1·135 × 0·342 mm.). Cuticle set with rows of minute backwardly-directed spines as far back as the acetabulum ("gonotyl" of Witenberg); behind this the spines are fewer and gradually disappear.

Oral sucker subterminal, 0·059–0·081 by 0·074–0·092 mm. (av. 0·069 by 0·071). Prepharynx, 0·055–0·074 mm. Pharynx barrel-shaped, 0·055–0·074 by 0·037–0·055 mm. Oesophagus 0·044–0·055 mm. long. Caeca terminating near posterior end of body. Nerve ring 0·09 mm. from head end.

Genital sac at about middle of body, oval, 0·074–0·137 long by 0·082–0·155 mm. wide (av. 0·097–0·103 mm.). Gonotyl spines in a comma-shaped group with about 40 narrow, slightly curved spines, 0·015 mm. long, in a circular group from which extends a semicircle containing 20–30 spines in an irregular row, gradually shortening to 0·008 mm. at the distal end (Figs 10–12). Ventral sucker vestigial, at anterior edge of genital sac.

Testes ovoid, parallel or slightly oblique, smooth outline, 0·078 × 0·092 mm., just inside caeca, at posterior part of middle third of body. Vesicula seminalis voluminous, constricted into three parts between ovary and gonotyl. Walls of ejaculatory duct thickened. Male and female ducts open into genital sinus (Figs 13, 14).

Ovary transversely elongated, oval, sometimes slightly lobed, 0·055–0·070 × 0·074–0·081 mm., just in front of right testes; oviduct originates from median posterior edge and passes through ootype to descending uterus. Uterine loops fill whole of body behind ovary extending laterally beyond the intestinal caeca. Ascending uterus makes a transverse loop in front of testes and continues to the genital pore. Eggs elliptical to oval, brown, average 0·034 × 0·019 mm., embryonated, some with prominent abopercular knob. Vitellaria acinous, in posterior third of body, mostly inside post-testicular intestinal caeca. Seminal receptacle between testes.

Stictodora specimens from feeding experiments A to F were compared and were all regarded as belonging to one species. The same species was also found as a natural infection in 3 of 7 mature *Larus novae-hollandiae* collected from Five Islands, near Sydney. This is a breeding site for gulls which normally live and feed at Narrabeen and other coastal lagoons where they would be exposed to infection by cercariae from *Velacumantus*.

Discussion.

Species of *Stictodora* previously reported from Australia are *S. diplacantha* Johnston. 1942, from a cormorant, *Phalacrocorax varius*, and *S. lari* Yamaguti, 1939, from *Pelecanus conspicillatus* and the domestic cat (Pearson, 1960).* Pearson and the writer have compared the last-named specimens with those recovered from experimental gulls and cats in Sydney and agree that they are the same species.

The genus was named and defined by Looss (1899) with the type species *S. sawakinensis*; revisions of the generic description have been made by Witenberg (1929, 1953) and Yamaguti (1939). Chen (1951) tabulates the characters of eight species, Witenberg (1953) has a key to 10 species, and Yamaguti (1958) lists 14 species. The type host of *S. lari* is *Larus crassirostris* Vieillot from Japan.

The most important features defining species of *Stictodora* are those of the genital sac and gonotyl, especially the number, arrangement and shape of the gonotyl spines. In the present study, some variation in the arrangement of spines was noted even in specimens from one experimental host (Figs 10, 11, 12 from gull A). The variations may be due to coverslip pressure during fixation; living specimens under light pressure generally appear as in Figure 10.

Witenberg's (1953) drawing of the gonotyl spines of *S. guerreroi* Garcia and Refuerzo, 1936, resembles Figure 12 except that he shows twice as many spines. This also applies to *S. japonica* Yamaguti, 1939. Exact counts of the gonotyl spines are usually difficult, but can be estimated closely, and the numbers lie between 50 and 80 in my own material.

Members of the family Heterophyidae use fish-eating birds as main hosts, but can maintain themselves in a variety of mammals which eat raw fish, notably cats, dogs and other carnivores, and man. Cercariae develop in operculate snails and encyst as metacercariae in several species of fish, perhaps belonging to different families. *Stictodora lari* shows no exceptional features from this general pattern of development.

Little is known of specific or generic differences in the cercariae of Heterophyidae and it is possible that more than one species are present in *V. australis*. Evidence against this is provided by the absence of other trematodes in cats and gulls fed with several kinds of small fish from the weedbeds.

Birds other than the silver gull have not been examined for *Stictodora* in Sydney area, but in Brisbane Pearson (1960) found them in pelicans and reared them in cats. The wide host ranges of heterophyids suggest that other species of aquatic birds would be infected. Migrating species coming from north-east Asia are common during summer on littoral Australia and bring many parasite species with them. A small number of them were examined at Townsville, North Queensland, but no species of Heterophyidae was found. Migrant species available and their numbers were: *Limosa lapponica*, 7;

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* Dr. Pearson reported these parasites as *S. caballeroi* Martin, 1955. He has recently compared type material of this species and of *S. lari* Yamaguti, 1939, and now regards them as synonymous (personal communication, 1961).

Charadrius leschenaultii, 3; *C. alexandrinus*, 1; *Calidris tenuirostris*, 3; *C. canuta*, 2; *Tringa hypoleuca*, 1. The diets of these birds are probably small insects and crustaceans, and not fish. Eight silver gulls and four terns (*Sterna bergii*) from the same locality had Heterophyidae, but none of the genus *Stictodora*. In this latitude (19° 5′ south) estuarine flats are covered with mud and mangroves, and *Velacumantus* does not occur.

Slides of adult *S. lari* from this investigation are stored in the parasite collection of this School numbered Mn 1380, 1381, 1385, 1488, 1489; metacercariae and cercariae are in formalin, numbered Mn 1386.

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